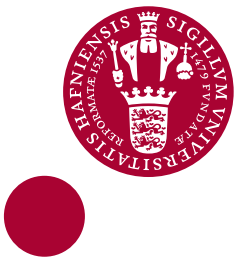

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The influence of light and water mass on bacterial population dynamics in the Amundsen Sea Polynya

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Abstract

Despite being perpetually cold, seasonally ice-covered and dark, the coastal Southern Ocean is highly productive and harbors a diverse microbiota. During the austral summer, ice-free coastal patches (or polynyas) form, exposing pelagic organisms to sunlight, triggering intense phytoplankton blooms. This strong seasonality is likely to influence bacterioplankton community composition (BCC). For the most part, we do not fully understand the environmental drivers controlling high-latitude BCC and the biogeochemical cycles they mediate. In this study, the Amundsen Sea Polynya was used as a model system to investigate important environmental factors that shape the coastal Southern Ocean microbiota. Population dynamics in terms of occurrence and activity of abundant taxa was studied in both environmental samples and microcosm experiments by using 454 pyrosequencing of 16S rRNA genes. We found that the BCC in the photic epipelagic zone had low richness, with dominant bacterial populations being related to taxa known to benefit from high organic carbon and nutrient loads (copiotrophs). In contrast, the BCC in deeper mesopelagic water masses had higher richness, featuring taxa known to benefit from low organic carbon and nutrient loads (oligotrophs). Incubation experiments indicated that direct impacts of light and competition for organic nutrients are two important factors shaping BCC in the Amundsen Sea Polynya.

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Introduction

Despite the cold conditions, heterotrophic bacterioplankton communities are thriving in the Southern Ocean (SO). These cold-adapted microbes mediate the transformation and remineralization of organic and inorganic nutrients and contribute significantly to elemental cycles and to the marine carbon pump. Numerous cold-water studies have measured rates of bacterial activity similar to those measured in temperate oceanic regions (e.g., Cota et al., 1990; Granéli et al., 2004; Williams et al., 2014). Hence, polar marine bacterioplankton have a central role in these ecosystems, yet it remains challenging to elucidate how the combination of habitat-specific drivers affects the growth, distribution and eventually functional role of individual populations in these cold oceanic regions.

Spatial partitioning caused by hydrographical separation and contrasting light conditions (photic/aphotic) likely select for bacterioplankton populations carrying specific traits with regards to energy and nutrient acquisition, as well as predation resistance and many other metabolic functions (Pernthaler, 2005;

Violle et al., 2007; Comte and del Giorgio, 2011). In surface waters across the SO, seasonally recurring but patchy summer areas of open water also promote biological activity because of the increased surface water irradiance. Extremely productive phytoplankton blooms, fueled by a combination of this higher radiation, enhanced stratification from sea ice meltwater, and nutrient supply from bottom water interaction with the continental ice sheet (Sherrell et al., 2014), are typical for these polar waters and affect the entire food web (Ducklow et al., 2001; Arrigo and Dijken, 2003; Alderkamp et al., 2012). Transient and patchy inputs of phytoplankton-derived organic substrates from such blooms drastically change the bottom-up factors controlling heterotrophic bacterioplankton (Billen et al., 1990) and likely also promote shifts in their abundance and community structure.

Light can directly influence the growth of several bacterial groups that are able to sustain, or at least supplement, their energy demands by harvesting photons (Bryant and Frigaard, 2006). Besides the canonical oxygenic photosynthesis characteristic for cyanobacteria and eukaryotic phytoplankton, some bacterial phototrophs are capable of anaerobic anoxygenic photosynthesis (e.g., *Rhodobacteraceae*), or proteorhodopsin-mediated energy harvesting (e.g., *Polaribacter* [Cottrell and Kirchman, 2009; Koh et al., 2010], SAR11 [Giovannoni et al., 2005] and some *Gammaproteobacteria* [Stingl et al., 2007]). However, direct exposure to high solar radiation is also known to inhibit the growth of some heterotrophic populations (Doudney and Young, 1962; Okubo and Nakayama, 1967; Cabiscol et al., 2010). Thus, the dramatic increase in light intensity during austral summer is likely to cause a rapid shift in surface-water community structure by favoring taxa that harvest light at the expense of those that are susceptible to photoinhibition.

Hydrographic separation can partition and isolate bacterioplankton communities, as observed in the North Atlantic (Agogué et al., 2011) and in polar oceans (Galand et al., 2010; Alonso-Sáez et al., 2011; Hamdan et al., 2013), but also in more global surveys of marine bacterioplankton (Ghiglione et al., 2012). Hydrographic separation was recently recognized to be a common feature separating the bacterioplankton communities in the Southern Ocean (Wilkins et al., 2012). The Antarctic shelf region that encompasses the Amundsen Sea Polynya (ASP; the open-water area of this study), can be separated into three water masses during summer: at depth is the warmer but more saline modified Circumpolar Deep Water (mCDW), overlain by the colder and less saline Winter Water (WW) and by the warmer and less saline Antarctic Surface Water (AASW), which is influenced by freshwater from melting sea ice and present only temporarily during austral summer in close connection to the ice retreat (Randall-Goodwin et al., 2014).

In the present study, the remote ASP was used as a model-system to consider how light and water mass interactively influence the composition of local bacterioplankton communities. We carried out experiments during the Amundsen Sea International Research Expedition (ASPIRE, Yager et al., 2012) with the broader aim of assessing how individual bacterial taxa respond to light, by experimentally simulating two contrasting light regimes experienced by bacterioplankton in this region during the course of the year. Communities emerging in experimental incubations were also compared to the communities residing in the different water masses that characterize the ASP. Two complementary datasets of ASP bacterioplankton community composition were collected by use of 454 pyrosequencing of amplified 16S rRNA gene fragments. Field observations across the different water masses were combined with a factorial experiment where bacterioplankton communities from the different water masses were exposed to darkness and to photic-zone levels of irradiation. Focus was on the distribution and responses of a subset of dominant bacterial groups present in the ASP. These previously studied groups with inferred metabolic traits were highlighted to identify and illustrate individual and contrasting population-level responses to light regime and water mass. The underlying hypothesis was that differing light conditions and other distinctions between water masses select for particular metabolic traits, resulting in the emergence of microbial populations specifically adapted to these local conditions.

Methods

Sampling

Sampling was conducted during the austral summer (November 2010 to January 2011) from the icebreaker Nathaniel B. Palmer. Samples from 15 stations were obtained to include samples from the three major water masses of the Amundsen Sea Polynya and its margins (71–75°S, 110–120°W; Figure 1) during the summer season. Seawater was collected in 12 L Niskin bottles attached to a 24-bottle SBE 32 rosette; coupled to the rosette was a system of sensors reading depth-resolved profiles of temperature [° C], conductivity [S m^{-1}], oxygen [mg L^{-1}], photosynthetically active radiation (PAR) [µmol photons s^{-1} m^{-2}] and fluorescence [mg m^{-3} chl-a] for each cast (SBE 911, Sea-Bird Electronics, Bellevue, Washington, USA). Water samples for incubation experiments were processed immediately at 2°C in a temperature-controlled room.

Bacterial community analysis

Bacteria from sampled seawater were collected by filtration onto 0.2 µm membranes in Sterivex filter-cartridges (Millipore, Solna, Sweden) using peristaltic pumps and acid-washed silicone tubing. For each of 2–5 depths

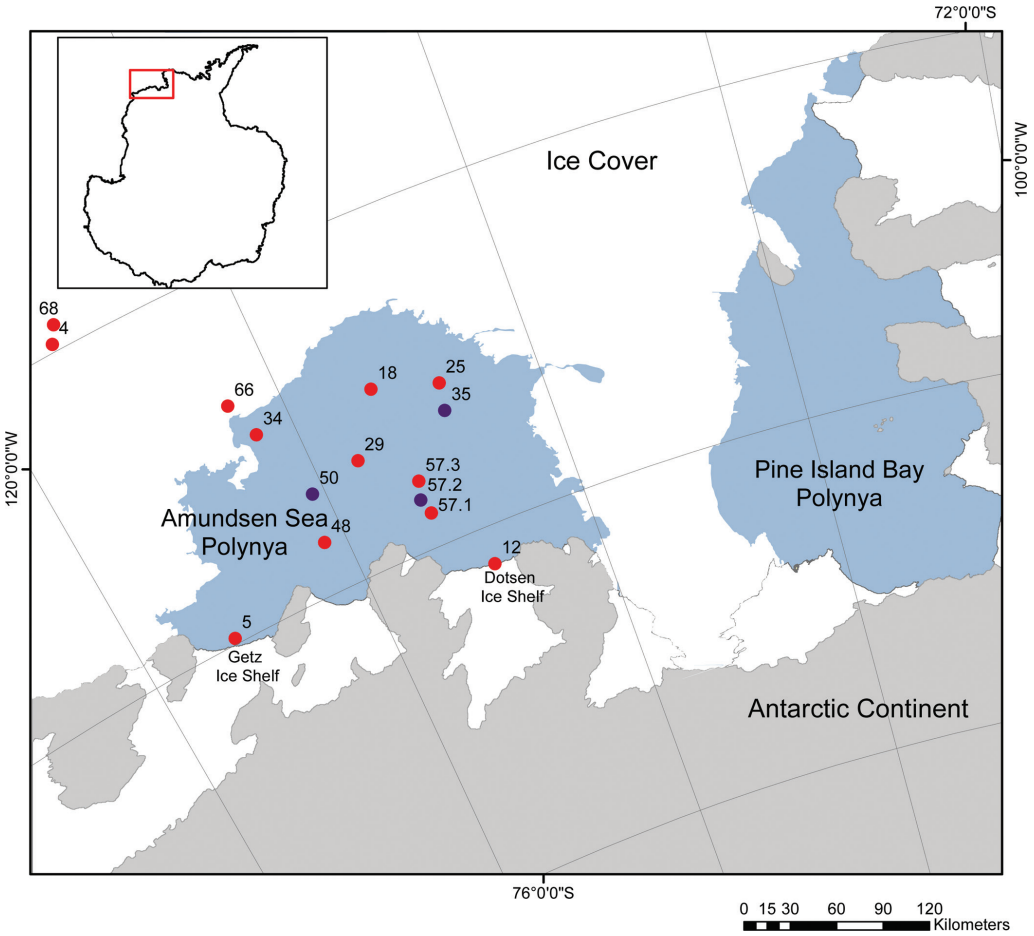

Figure 1

Map of the Amundsen Sea Polynya and stations sampled.

Map illustrating the extent of seasonal ice retreat in the Amundsen Sea at the time of sampling. Stations sampled to examine *in situ* bacterial distributions are marked by solid circles (red and purple). The seawater inocula and media for the shipboard incubations were obtained from stations marked in purple (st35, st50, and st57.2).

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per station, a volume of approximately 5 L of water was filtered. The filters were subsequently covered with a sucrose lysis buffer (20% sucrose, 50 mM EDTA, 50 mM Tris HCL, pH 8) and stored at −80°C.

Incubation experiments

To assess bacterioplankton responses to light and dark conditions in the absence of larger predators and eukaryotic phytoplankton, 0.2 µm filtered seawater in 1 L acid washed polycarbonate bottles was inoculated with 5% [v/v] 0.6 µm filtered seawater from the same depth using a vacuum pump to a total volume of 1 L. The experimental design by station included two factors (water mass source of inoculum: epipelagic and mesopelagic) with two treatments (dark and light) for each inoculum. Triplicate incubations were conducted under the dark and light conditions using water from each of three stations: 35 (73°27′95″S, 112°10′41″W), 50 (73° 41′60″S, 115°25′03″W) and 57.2 (73°70′73″S, 113°26′5″W) (Figure 1). Each experiment included one inoculum from the light-exposed AASW, and one from the mesopelagic zone from either WW or mCDW. Each 0.2-µm filtered seawater medium came from the same depth and station as its inoculum. The light source imitated light levels at approximately 20–50 m below the surface (Philips TLD-18W/18 blue, 1.5–1.99 $^{*}10^{-2}$ µmol photons s^{-1} m^{-2}) in a PAR range of 400–500 nm (according to manufacturer), excluding the short-wavelength UV. After a 7-day incubation at near in situ temperature (0.5°C), bacteria from the full sample volume were collected by vacuum filtration onto 0.2-µm, 47-mm Supor filters (Pall, Lund, Sweden) and stored at −80°C in sucrose lysis buffer (20% sucrose, 50 mM EDTA, 50 mM TrisHCl, pH = 8). Darkness was achieved by covering the bottles with black and lightproof foil.

Bacterial abundance

For measuring *in situ* bacterial abundance and bacterial abundance in the incubation experiments, 1.5 ml water was sub-sampled at time zero and three subsequent occasions during the incubation and fixed in 1% EM grade glutaraldehyde (Sigma Aldrich), flash-frozen in liquid nitrogen, and stored at −80°C. Bacterial abundance was determined with a FASCanto II flow cytometer (Becton Dickinson, USA) (Gasol and del

Giorgio, 2000) after staining the fixed cells with SYBR green (Invitrogen). The flow rate was calibrated with fluorescent beads. Net growth was determined for each incubation by the change in bacterial abundance after 7 days, relative to the control (time zero), and reported as the mean percentage for the triplicate experiments.

Molecular analysis

The DNA was extracted using a phenol-chloroform extraction approach as previously described (Riemann et al., 2000). Prior to extraction, microorganisms were enzymatically digested for 30 min with lysozyme at 37°C followed by an overnight digestion with Proteinase K (both 20 mg ml^{-1}, Sigma Aldrich) at 55°C (Boström et al., 2004). The 16S rRNA genes were amplified using the bacterial primers Bakt_341F (CCTACGGGNGGCWGCAG) and Bakt_805R (GACTACHVGGGTATCTAATCC) with 454-Lib-L adapters and sample-specific barcodes on the reverse primer (Herlemann et al., 2011). Each set consisted of up to 72 samples with individual barcodes pooled for sequencing (Table 1). Triplicate PCR reactions for each sample were carried out with 10 to 70 ng extracted environmental DNA as template. Each 20-µl reaction also contained Phusion Hot Start high-fidelity DNA polymerase (Thermo Scientific). Amplification was carried out by initial denaturation at 98°C for 30 seconds followed by 25 cycles of an initial 98°C denaturation for 30 seconds, subsequent annealing at 50°C for 30 seconds and 30-second extension at 72°C. These 25 cycles were followed by a final 7-min extension at 72°C. Triplicate reactions for each sample were pooled and PCR products were purified using the Agencourt AMPure XP kit according to manufacturer instructions (Beckman Coulter) and quantified with a Picogreen quantification essay (Invitrogen). Equimolar amounts of amplicon from each sample were pooled and sequenced by 454 pyrosequencing using Titanium chemistry at the SNP/SEQ SciLifeLab platform hosted by Uppsala University (Sweden).

Table 1. Individual barcode sequences used in the two 16S rRNA amplicon batches

Primer[a] ID	Barcode	Batch 1	Batch 2
		Station_depth (m)_ Treatment	
4	TATCGCA	57/cast71_30	
5	TACTAGC	4_638	
6	TACTCTC	4_2	
7	TACTCGA	4_25	
8	TACTGAC	4_70	
9	TACTGCA	4_560	
10	TACGTCA	5_2	
11	TACGAGT	5_25	35_120_dark
12	TACGCTA	18_20	35_120_dark
13	TAGTCAC	5_750	35_120 dark
14	TAGACTC	5_1227	35_12_dark
15	TAGACGA	12_22	35_12_dark
16	TAGAGAC	12_80	35_12_dark
17	TAGAGCA	12_240	35_120_light
18	TAGCTCA	12_600	35_120_light
19	TAGCACT	12_900	35_120_light
20	TAGCAGA		35_12_light
21	TAGCGTA	5_70	35_12_light
22	TCTACTC	18_50	35_12_light
23	TCTCTCA	18_350	50_120_dark
24	TCTCATC	18_422	50_120_dark
25	TCTCACT	25_2	50_120_dark
26	TCTCAGA	25_18	50_10_dark
27	TCTGAGT	25_80	50_10_dark
28	TCATAGC	25_350	50_10_dark
29	TCATCTC	25_400	50_120_light
30	TCATCGA	12_180	50_120_light
31	TCATGAC	29_2	50_120_light

Primer[a] ID	Barcode	Batch 1	Batch 2
		Station_depth (m)_ Treatment	
32	TCATGCA	29_20	50_10_light
33	TCACTAC	29_80	50_10_light
34	TCACTCT	29_655	50_10_light
35	TCACTGA	29_733	57/cast72_680_dark
36	TCACACA	34_2	57/cast72_680_dark
37	TCACAGT	34_10	57/cast72_680 dark
38	TCACGTA	34_50	57/cast72_10_dark
39	TCACGAT	34_360	57/cast72_10_dark
40	TCAGTCA	34_672	57/cast72_10_dark
41	TCAGATC	35_2	57/cast72_680_light
42	TCAGAGA	35_12	57/cast72_680_light
43	TCAGCTA	35_120	57/cast72_680_light
44	TCGTAGA	35_360	57/cast72_10_light
45	TCGTGTA	35_420	57/cast72_10_light
46	TCGATCA		57/cast72_10_light
47	TCGACTA	48_2	
46	TCGATCA	48_25	
48	TCGCATA	48_120	
49	TGTACGA	48_500	
50	TGTAGCA	48_983	
51	TGTCACA	50_5	
52	TGTCGTA	50_10	
53	TGTGTCA	50_170	
54	TGTGCTA	50_320	
55	TGATCAC	50_1031	
56	TGACTCA	57/cast71_4	
57	TGACACT	57/cast71_140	
58	TGAGTAC	57/cast71_500	
59	TGAGTCT	57/cast71_735	
61	TGAGCAT	57/cast72_10	
62	TGCTAGA	57/cast72_150	
63	TGCTGTA	57/cast72_300	
64	TGCATCA	57/cast72_772	
65	TGCACTA	57/cast72_680	
66	TGCGATA	57/cast84_5	
67	ATACTGC	57/cast84_10	
68	ATACGCT	57/cast84_150	
69	ATAGCGT	57/cast84_300	
70	ATCTCAC	57/cast84_625	
71	ATCATGC	66_2	
72	ATCACTC	66_10	
73	ATCACGT	66_100	
74	ATCAGAC	66_636	
75	ATCAGCT	68_25	
76	ATCGTGT	68_500	
79	ATGTCGT	68_820	

[a] Reverse Primer 805R with Titanium Adapter A

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In order to obtain a list of observed operational taxonomic units (OTUs) suitable for statistical analysis, low-quality sequences were removed from the dataset, and noise was reduced using AmpliconNoise v1.24 (Quince et al., 2011) with default parameters. AmpliconNoise implements algorithms that remove PCR single-base and 454-pyrosequencing errors, as well as the chimera removal tool Perseus. Reads that did not carry the exact primer sequence were removed. With a length-cutoff of 425 base pairs (bp), the remaining reads were processed using the Quantitative Insights Into Microbial Ecology software (QIIME v1.3, Caporaso et al., 2010). Sequences were clustered into OTUs at 99% pairwise identity using Uclust (Edgar, 2010). Taxonomic assignments of representative sequences from each OTU were obtained according to the SILVA111 database (Quast et al., 2013) by using the RDP classifier implemented in QIIME (Wang et al., 2007). After excluding non-bacterial taxa and singletons, altogether 488,028 reads classified into 452 OTUs, which were kept for further analysis.

Statistical analysis

The curated OTU table generated after our quality control was used to calculate alpha diversity (Simpson diversity, Shannon diversity, observed OTUs, Chao1 richness) with QIIME. Reads obtained for each OTU were transformed into relative abundances across samples. For alpha diversity, 2,000 reads were subsampled by rarefaction in order to reduce any possibility of biases due to uneven sequencing efforts across samples. Samples with less reads were excluded.

For analyzing the *in situ* populations, first the degree of group separation was tested statistically by Analysis of Similarity (ANOSIM) in RStudio. In a following step SIMPER (Similarity Percentage) analysis was conducted using Primer6 (Clarke, 1993; Clarke and Warwick, 2001), based on Bray Curtis dissimilarity, to identify the OTUs responsible for significant differences in community composition across the different water masses. The individual samples were eventually grouped by their water mass of origin based on their temperature and salinity signatures (Randall-Goodwin et al., 2014).

The experimental incubations were evaluated with regards to the responses in alpha diversity and net-growth of the total community, and to population-level responses for specifically targeted populations. It was hypothesized that: (i) light will favor phototrophic or photoheterotrophic bacterial populations and (ii) the origin of the water sample and inoculum will generate differences in the emergent communities because of a water-mass-specific species pool and contrasting local nutrients and organic resources available in the filtered seawater media.

For statistical analysis, experimental data were checked for normality and homogeneity of variance before applying parametric tests. Response in terms of net-growth (increase in bacterial abundance relative to initial bacterial concentration), Simpson diversity, Shannon diversity and Chao1 richness across the four treatments was assessed with a two-way ANOVA testing for the factors light and epipelagic/mesopelagic water mass. All of these analyses were carried out in RStudio. The Simpson diversity estimator and Shannon diversity indices for alpha diversity applied here were calculated based on the proportional abundance of taxa; in comparison, the Simpson diversity is more sensitive to changes in dominant taxa than the rare ones. The estimator applied for capturing richness was Chao1, accounting for rare taxa that might have been missed due to the sequencing approach (see Hill et al., 2003, for an overview of alpha diversity metrics).

Population dynamics analysis

To assess the response of individual taxonomically defined populations rather than the combined and rather complex communities, a subset of abundant bacterial populations (defined by OTUs classified at the genus level) was selected for further analysis. This subset was chosen to illustrate population dynamics under different environmental conditions using examples of populations with contrasting metabolic features. The examples represent key organisms within the bacterioplankton community of the Southern Ocean, the metabolic features and ecology of which have been highlighted and discussed in several recent publications (Koh et al., 2010; Williams et al., 2012; Tripp, 2013). The selected genera were identified by SIMPER to contribute substantially to the observed differences in community composition between the water masses. They were comprised of pairs of closely related bacterial taxa selected from each of four broader taxonomic groups: the *Alphaproteobacteria* (*Roseobacter* and SAR 11), the *Flavobacteriales* of the *Bacteriodetes* (*Ulvibacter* and *Polaribacter*), and the *Oceanospirillales* (*Balneatrix* and SAR86) and *Alteromonadales* (*Colwellia* and SAR92) within the *Gammaproteobacteria* (according to the Silva111 reference database). We did not test for significance here as the low relative abundance might cause a bias.

Results

In situ bacterial community composition of the water masses

Characteristics of the samples collected for the *in situ* survey of bacterioplankton communities, which allowed water mass origin to be confirmed, are summarized in Table 2. Bacterial communities differed

Table 2. Characteristics of samples collected to survey *in situ* bacterial populations[a]

Station[a]	Latitude (°S)	Longitude (°W)	Depth (m)	Water mass	Salinity	Temperature (°C)	Total bacteria (number x 10^5 ml^{-1})	Simpson Diversity	Shannon Diversity	Chao1 richness	Observed taxa
4	71°95′29″	118°47′26	70	WW	34.03	−1.80	2.18	0.90	4.13	79	70
4	71°95′29″	118°47′26	638	mCDW	34.66	0.81	0.66	0.90	4.30	126	98
5	73°96′66″	118°03′48″	2	WW	33.99	−1.41	4.70	0.81	3.19	79	57
5	73°96′66″	118°03′48″	25	WW	33.99	−1.69	4.83	0.82	3.19	63	51
5	73°96′66″	118°03′48″	750	mCDW	34.53	0.59	0.88	0.90	4.41	115	95
5	73°96′66″	118°03′48″	1227	mCDW	34.58	0.79	0.96	0.89	4.27	133	99
12	74°21′85″	112°33′62″	240	WW	34.03	−1.26	1.27	0.93	4.73	110	92
12	74°21′85″	112°33′62″	600	mCDW	34.41	0.27	1.18	0.88	4.10	112	86
12	74°21′85″	112°33′62″	900	mCDW	34.55	0.60	1.09	0.87	4.18	115	90
18	73°	113°30′23″	20	WW	33.95	−1.26	7.97	0.73	2.56	73	47
18	73°	113°30′23″	50	WW	34.02	−1.72	6.45	0.84	3.44	74	56
18	73°	113°30′23″	350	WW	34.31	−0.30	1.25	0.89	4.35	117	94
18	73°	113°30′23″	422	mCDW	34.52	0.43	1.44	0.81	3.79	109	87
25	73°12′01″	112°00′07″	2	AASW	33.83	−0.61	4.22	0.72	2.68	66	48
25	73°12′01″	112°00′07″	18	AASW	33.89	−1.13	6.00	0.76	2.77	54	43
25	73°12′01″	112°00′07″	350	WW	34.30	−0.42	1.12	0.86	4.21	116	95
25	73°12′01″	112°00′07″	400	mCDW	34.44	0.22	1.30	0.84	4.06	106	90
29	73°35′04″	114°12′68″	2	AASW	33.91	−0.90	4.99	0.56	1.82	54	36
29	73°35′04″	114°12′68″	20	AASW	33.91	−0.92	5.36	0.65	2.14	56	39
29	73°35′04″	114°12′68″	80	WW	34.03	−1.62	2.87	0.83	3.27	70	54
29	73°35′04″	114°12′68″	655	mCDW	34.52	0.48	1.33	0.87	4.15	122	97
29	73°35′04″	114°12′68″	733	mCDW	34.62	0.75	1.59	0.85	3.94	106	87
34	72°96′35″	115°75′96″	2	AASW	33.74	−1.07	4.56	0.74	2.69	66	49
34	72°96′35″	115°75′96″	10	AASW	33.76	−1.25	4.56	0.70	2.60	62	50
34	72°96′35″	115°75′96″	50	WW	34.00	−1.72	4.98	0.88	3.71	73	52
35	73°27′95″	112°10′41″	2	AASW	33.83	−0.17	4.53	0.73	2.60	74	42
35	73°27′95″	112°10′41″	12[b]	AASW	33.83	−0.20	5.04	0.67	2.31	47	40
35	73°27′95″	112°10′41″	420	mCDW	34.45	0.25	1.57	0.90	4.41	105	89
48	73°70′13″	115°44′99″	2	AASW	33.89	−0.18	2.54	0.70	2.22	36	29
48	73°70′13″	115°44′99″	120	WW	34.04	−1.74	1.71	0.87	3.54	56	51
48	73°70′13″	115°44′99″	500	mCDW	34.35	−0.26	1.14	0.92	4.73	115	96
48	73°70′13″	115°44′99″	983	mCDW	34.57	0.67	1.18	0.85	4.13	122	96
50	73°41′61″	115°25′03″	5	AASW	33.87	−0.33	5.11	0.63	2.19	58	37
50	73°41′61″	115°25′03″	10[b]	AASW	33.88	−0.37	4.49	0.65	2.17	48	33
50	73°41′61″	115°25′03″	320	WW	34.13	−1.74	1.62	0.94	4.95	110	89
57.1	73°80′17	113°16′5″	4	AASW	33.93	−0.50	4.35	0.57	1.88	38	30
57.1	73°80′17	113°16′5″	30	AASW	33.93	−0.53	4.34	0.51	1.78	40	33
57.1	73°80′17	113°16′5″	140	WW	33.98	−1.38	2.99	0.84	3.61	105	75
57.2	73°70′73″	113°26′53″	10[b]	AASW	33.93	−0.50	3.80	0.69	2.62	67	49
57.2	73°70′73″	113°26′53″	772	mCDW	34.59	0.71	missing	0.87	4.14	111	91
57.3	73°60′22″	113°14°88″	5	AASW	33.92	−0.26	4.52	0.70	2.40	42	33
57.3	73°60′22″	113°14°88″	10	AASW	33.92	−0.28	3.99	0.71	2.40	45	36
57.3	73°60′22″	113°14°88″	150	WW	34.05	−1.17	3.09	0.88	3.73	62	57
66	72°74′09	116°01′99″	2	AASW	33.60	−1.17	4.15	0.80	2.96	67	43
66	72°74′09	116°01′99″	10	AASW	33.69	−1.28	4.35	0.82	3.06	68	43
66	72°74′09	116°01′99″	100	WW	34.07	−1.61	2.96	0.86	3.69	80	63

[a] Only samples with read counts > 2000 were included
[b] These samples, along with three others not listed here, were used for inocula and media in the incubation experiments
doi: 10.12952/journal.elementa.000044.t002

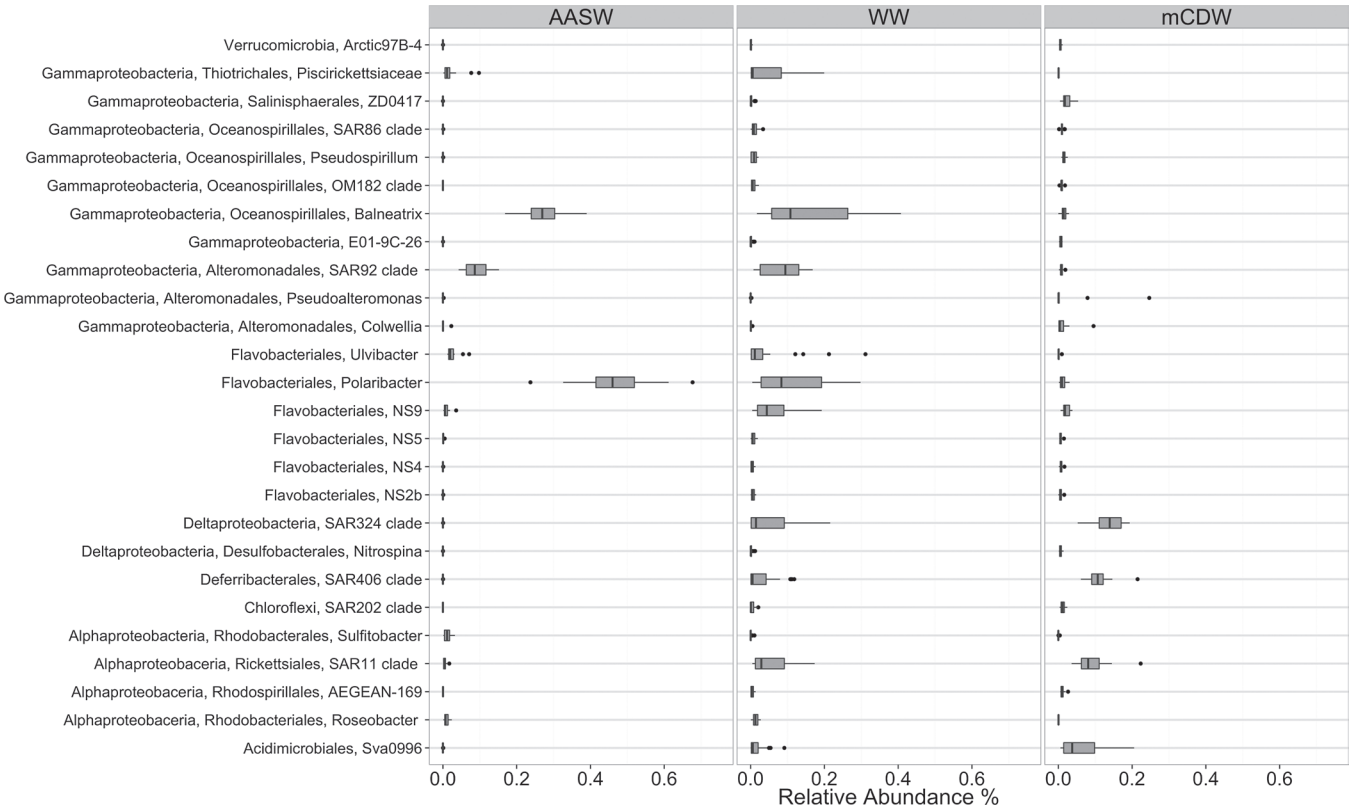

Figure 2

Relative abundance of bacterial taxa across the water masses.

Bacterial genus-level taxa identified by Similarity Percentage (SIMPER) analysis to explain 90% of the Bray-Curtis dissimilarities between pairs of sampled water masses. Multiple OTUs affiliated with the same taxon were combined for the analysis and visualization. For each taxon, boxplots illustrate the 25–75 percentile values (box), median (bar inside box), standard deviation (whiskers), and outliers (solid circles).

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significantly between the three water masses (AASW, WW, and mCDW) according to the ANOSIM analysis, even though the R values pointed to different degrees of separation, with R = 0.28 for AASW and WW, R = 0.41 for WW and mCDW, and R = 0.87 for AASW and mCDW (p = 0.001 for all three cases). According to SIMPER, about 30 OTUs alone explained 90% of the dissimilarity in bacterial community composition between each of the three water masses (Figure 2). AASW was characterized by low species richness caused by the dominance of *Flavobacteria/Polaribacter* and *Oceanospirillaceae/Balneatrix* clades with a similarity of 74% within the group. The observed dissimilarity in community composition between WW and mCDW was mainly due to shifts in the relative abundances of broadly distributed taxa, but mCDW hosted additional abundant taxa that were rare in both AASW and WW. For example, *Deltaproteobacteria* clade SAR324, *Deferribacterales* clade SAR 406 and *Acidomicrobiales* clade Sva0996 were prevalent bacterial community members in mCDW. SIMPER analysis further identified that the bacterioplankton community in mCDW was more homogenous in composition than in WW with a within-group similarity of 65% and 53%, respectively. Alpha diversity differed between the water masses, in that mCDW and WW harbored more diverse bacterioplankton communities than AASW (Table 3). Total bacterial abundance was highest in AASW, averaging 4.4 (± 0.6) × 10^5 bacteria ml^{-1}, whereas in WW and mCDW, abundance averaged 2.8 (± 1.8) × 10^5 and 1.2 (± 0.3) × 10^5 bacteria ml^{-1}, respectively (Figure 3).

Experimental treatments

At the time of sampling, bacterial inocula originating from the epipelagic zone at 2–10 m had experienced PAR in the range of 5.6–105 µmol photons s^{-1} m^{-2}, while no PAR reached the mesopelagic zone or the bacteria used as inocula from this zone. Experimental incubations with inocula from the mesopelagic water

Table 3. Alpha diversity[a] in the three water masses of the summer Amundsen Sea Polynya

Water mass	Simpson diversity	Shannon diversity	Observed OTUs	Chao1 richness
AASW (n = 18)	0.68 ± 0.07	2.4 ± 0.37	40 ± 7	55 ± 12
WW (n = 15)	0.86 ± 0.05	3.75 ± 0.62	67 ± 18	86 ± 21
mCDW (n = 13)	0.87 ± 0.03	4.2 ± 0.23	92 ± 5	115 ± 8

[a] Alpha diversity metrics, with standard deviations, were calculated based on rarefaction, with 2000 reads per sample included

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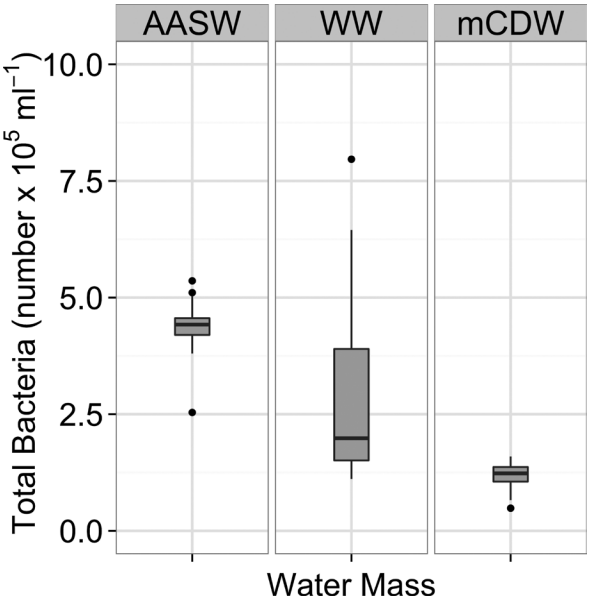

Figure 3

Total bacterial abundance in the water masses.

Bacterial cell counts derived from flow cytometry assays. The boxplots give the 25–75 percentile values (box), median (bar inside box), standard deviation (whiskers), and outliers (solid circles).

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Table 4. Characteristics[a] of bacterial communities in experimental incubations after 7 days

Sample origin[b]			Dark treatment					Light treatment				
Station	Depth (m)	Water mass	Simpson diversity	Shannon diversity	Chao1 richness	Observed OTUs	% net growth[c]	Simpson diversity	Shannon diversity	Chao1 richness	Observed OTUs	% net growth[c]
35	12	AASW	0.50 ± 0.12	1.47 ± 0.5	28 ± 11	22 ± 8	110 ± 92.3	0.3	2.31 ± 1.7	30.5	84 ± 8	17 ± 23.3
35	120	WW	0.90 ± 0.01	4.2 ± 0.02	82 ± 8	68 ± 3	13.7 ± 8.1	0.86 ± 0.02	3.9 ± 0.14	81 ± 3	65 ± 2	0.7 ± 1.2
50	10	AASW	0.40 ± 0.05	1.2 ± 0.13	22 ± 3	16 ± 2	152 ± 186	0.07 ± 0.02	0.31 ± 0.1	19 ± 10	12 ± 5	135 ± 117
50	120	WW	0.87 ± 0.01	3.7 ± 0.08	84 ± 6	65 ± 1	95.7 ± 135	0.41 ± 0.29	1.7 ± 1.23	56 ± 33	41 ± 23	40.5 ± 65.1
57.2	10	AASW	0.13 ± 0.09	0.52 ± 0.3	27 ± 3	19 ± 3	48.2 ± 25	0.14 ± 0.13	0.54 ± 0.4	30 ± 7	19 ± 3	3.6 ± 4.2
57.2	680	mCDW	0.85 ± 0.03	3.58 ± 0.2	88 ± 7	67 ± 5	50.6 ± 84.4	0.91[d]	4.39	87	82	0

[a] Alpha diversity metrics, with standard deviations, were calculated based on rarefaction, with 2000 reads per sample included; n = 3 except where indicated by missing standard deviation

[b] Seawater inocula and incubation media were taken from three different sites and two depths at each site to cover all three water masses in the ASP

[c] Net growth was calculated based on the difference in total bacterial abundance between time zero and 7 days

[d] Sequencing data retrieved only for one sample at this location

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masses generated the highest alpha diversity compared to those with inocula from the photic zone (AASW), while highest net growth was measured in dark treatments from AASW (Table 4). The two-factorial ANOVA of Simpson diversity as response variable for light and water mass demonstrated that water mass had a significant influence on this diversity index at all stations (Station 35: $F = 27.5$, $p = 0.002$; Station 50: $F = 16.8$, $p = 0.0046$; Station 57.2 $F = 139$, $p < 0.001$). Light was a significant factor only for Stations 50 ($F = 12.9$, $p = 0.008$) and 57.2 ($F = 7.3$, $p = 0.035$). When testing for Shannon diversity, the ANOVA revealed the same significant treatment effects as for Simpson diversity. Changes in species richness, represented by the Chao1 metric, were significantly affected by water mass at station 50 ($F = 18.6$, $p = 0.0035$) and 57.2 ($F = 241$, $p < 0.001$) and by light at station 57.2 ($F = 12.9$, $p = 0.011$). Despite considerable variation in growth among the incubations, there was a general trend across all three independent experiments that communities from the photic surface waters exhibited the highest net growth but also the greatest light inhibition (each experimental incubation; Tables 5, 6, 7).

Dynamics of a subset of populations in experimental treatments

Among the *Alphaproteobacteria* (Figure 4A), the SAR11 clade was a major community component in mCDW at 8.9 ± 4.4% and in WW at 5.2 ± 4.8%, but was insignificant in AASW (0.5 ± 0.5%). After the 7-day experimental incubations, SAR11 averaged 2.2 ± 2.5% and consistently represented an abundant community member in the incubated samples from WW and mCDW, but was rare in those from AASW, regardless of

Table 5. Results of experimental incubations with samples from station 35 (73°27′95″ S, 112°10′41″W)

Depth (m)	Water mass	Treatment	Total bacteria (number × 10^5 ml^{-1})				Simpson diversity	Shannon diversity	Chao1 richness	Observed taxa
			0 h	67 h	134 h	169 h				
120	WW	dark	0.09	0.05	0.09	0.10	0.90	4.16	73	65
120	WW	dark	0.08	0.06	0.09	0.09	0.90	4.19	86	67
120	WW	dark	0.09	0.09	0.09	0.10	0.91	4.21	88	72
12	AASW	dark	0.16	0.09	0.23	0.83	missing	missing	missing	missing
12	AASW	dark	0.24	0.28	0.41	1.23	0.58	1.82	36	28
12	AASW	dark	0.32	0.12	0.15	0.47	0.41	1.12	20	16
120	WW	light	0.07	0.06	0.05	0.06	0.84	3.79	81	64
120	WW	light	0.08	0.07	0.07	0.07	0.85	3.80	83	68
120	WW	light	0.08	0.08	0.09	0.08	0.89	4.03	77	64
12	AASW	light	0.57	0.12	0.14	0.29	missing	missing	missing	missing
12	AASW	light	0.35	0.10	0.14	0.27	0.66	3.49	182	143
12	AASW	light	0.15	0.13	0.28	0.12	0.30	1.13	31	25

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Table 6. Results of experimental incubations with samples from station 50 (73°41′60″ S, 115.25′03″ W)

Depth (m)	Water mass	Treatment	Total bacteria (number × 10^5 ml^{-1})				Simpson diversity	Shannon diversity	Chao1 richness	Observed taxa
			0 h	46 h	98 h	171 h				
120	WW	dark	0.06	0.06	0.41	0.70	0.86	3.72	80	64
120	WW	dark	0.02	0.03	0.08	0.03	0.87	3.83	88	65
10	AASW	dark	0.34	0.00	1.43	1.17	0.34	1.08	18	15
10	AASW	dark	0.11	0.23	0.74	1.43	0.45	1.34	24	18
10	AASW	dark	0.07	0.22	0.78	4.30	0.42	1.26	22	16
120	WW	light	0.03	0.03	0.03	0.04	0.67	2.86	74	59
120	WW	light	0.03	0.03	0.03	0.03	0.47	1.84	76	49
120	WW	light	0.03	0.04	0.03	0.27	0.10	0.42	18	15
10	AASW	light	0.06	0.13	0.43	1.44	0.05	0.21	8	7
10	AASW	light	0.05	1.02	0.41	0.92	0.07	0.33	27	16
10	AASW	light	0.05	0.13	0.00	1.59	0.09	0.39	21	13

doi: 10.12952/journal.elementa.000044.t006

Table 7. Results of experimental incubations with samples from station 57.2 (73°70′73″ S, 113.26′53″ W)

Depth (m)	Water mass	Treatment	Total bacteria (number × 10^5 ml^{-1})			Simpson diversity	Shannon diversity	Chao1 richness	Observed taxa
			0 h	56 h	173 h				
680	mCDW	dark	0.03	0.04	0.05	0.88	3.76	95	71
680	mCDW	dark	0.05	0.05	0.37	0.87	3.63	83	61
680	mCDW	dark	0.05	0.04	0.05	0.82	3.36	85	69
10	AASW	dark	0.63	0.25	1.02	0.24	0.86	25	19
10	AASW	dark	0.32	0.22	0.87	0.06	0.27	26	16
10	AASW	dark	0.50	0.15	0.29	0.09	0.45	31	22
680	mCDW	light[a]	0.03	0.03	0.03	missing	missing	missing	missing
680	mCDW	light	0.03	0.05	0.02	0.91	4.39	87	82
10	AASW	light	0.39	0.24	0.27	0.07	0.36	38	22
10	AASW	light	0.79	0.28	0.39	0.28	1.01	25	20
10	AASW	light	0.36	0.23	0.22	0.05	0.26	27	16

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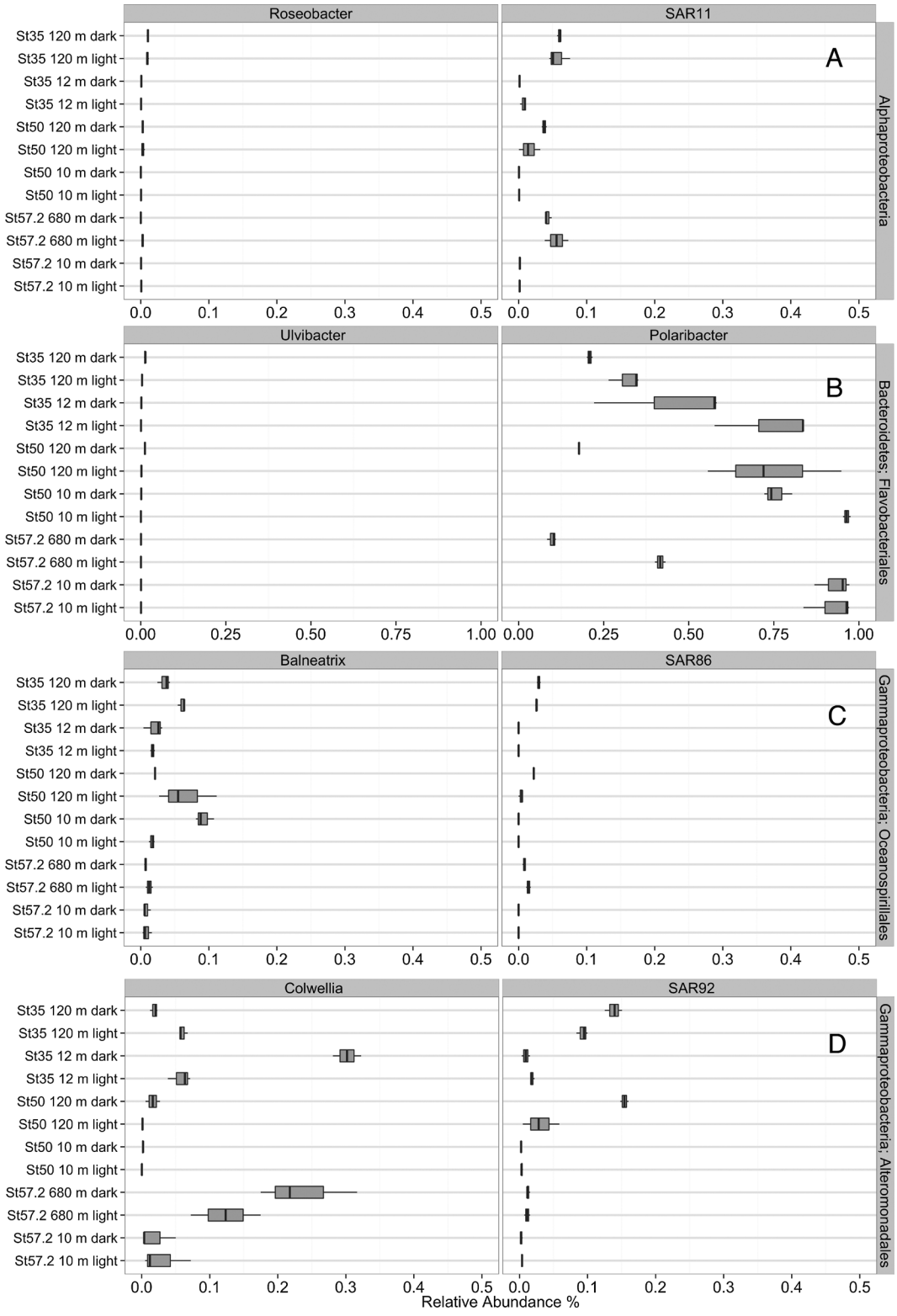

Figure 4

Distribution patterns of targeted bacterioplankton populations in incubation experiments.

Observed differences in the relative abundance of targeted populations at the end of the experimental incubations. Differences are plotted for each station (st35, st50, and st57.2) by water mass (where 10 and 12 m indicate AASW, 120 m indicates WW, and 680 m indicates mCDW) and by treatment (dark, light). Corresponding alpha diversity and net growth are given in Table 4. Targeted populations are (A) *Roseobacter* and SAR11 (*Alphaproteobacteria*), (B) *Ulvibacter* and *Polaribacter* (*Flavobacteriales* of the *Bacteriodetes*), (C) *Balneatrix* and SAR86 (*Oceanospirillales* of the *Gammaproteobacteria*), and (D) *Colwellia* and SAR92 (*Alteromonadales* of the *Gammaproteobacteria*). For each treatment, boxplots illustrate the 25–75 percentile values (box), median (bar inside box), and standard deviation (whiskers). No outliers were detected.

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light conditions. Similar to SAR11, members of the *Roseobacter* clade were scarce following treatments of water originating from AASW, despite the fact that *Roseobacter* was originally abundant in AASW at 1.0 ± 0.8% and in WW at 1.3 ± 0.8%. They were not significant in mCDW (0.05 ± 0.05%) and were reduced to 0.3 ± 0.4% in the incubated samples.

Within the *Flavobacteriales* (Figure 4B), the highly abundant *Polaribacter* contributed to the bacterioplankton communities in AASW at 45.7 ± 11.2%, in WW at 10.6 ± 9.4% and in mCDW at 1.2 ± 0.8%. *Polaribacter* reached a maximum average contribution of 57.7 ± 32% in the incubated samples, whereas the related *Ulvibacter* was much less abundant in each of the water masses and the incubated samples, with an average relative abundance of 2.5 ± 1.6% in AASW, 4 ± 7.5% in WW, 0.15 ± 0.2% in mCDW and 0.3 ± 0.4% in the incubated samples. *Ulvibacter* was nevertheless identified by SIMPER as one of the taxa contributing to the major part of the *in situ* community dissimilarity between water masses, but was clearly outcompeted in the incubations. In contrast *Polaribacter* was a dominant community member in all treatments, though it clearly preferred light-exposed treatments from WW and AASW.

Two representative clades from the *Oceanospirillales* (Figure 4C) within the *Gammaproteobacteria*, *Balneatrix* and SAR86, were selected for further scrutiny as they displayed contrasting abundance patterns. Of the total reads *Balneatrix* contributed 27.6 ± 6.25% in AASW, 15.6 ± 12.1% in WW, and 1.5 ± 0.7% in mCDW. It occurred in highest abundance in AASW and WW (similar to *Polaribacter*) but dropped following incubation to 3 ± 3%. SAR86 was present in all incubations, but at low relative abundances, averaging 0.8 ± 1.1%, similar to those observed in all of the water masses: 0.02 ± 0.03% in AASW, 1.1 ± 0.8% in WW, and 0.98 ± 0.41% in mCDW.

Within the *Alteromonadales* (Figure 4D), two ubiquitous marine clades, *Colwellia* and SAR92, displayed contrasting abundance patterns across the water masses. The contribution of *Colwellia* to the total communities was on average 0.2 ± 0.56% in AASW, 0.1 ± 0.1% in WW, and 1.2 ± 2.1% in mCDW. In the incubated samples, relative *Colwellia* abundance increased the most in samples from mCDW (8.5±14.8%) where its relative *in situ* abundance was also the greatest. In contrast, the average relative abundance of SAR92 was greater in both AASW at 8.9 ± 3.3% and WW at 8.1 ± 5.2% and lower in mCDW at 0.8 ± 0.4%, averaging 3.7 ± 5.2% following the incubations.

Discussion

Here we combined a spatial community survey of *in situ* bacterioplankton communities with controlled and replicated experimental incubations to illustrate how the environmental heterogeneity of the Amundsen Sea affects bacterioplankton population dynamics and community structure. Our results demonstrated that a relatively small number of taxa are responsible for the majority of observed dissimilarities in bacterial community composition between the three summer water masses of the Amundsen Sea Polynya, with observed differences originating mainly from shifts in the relative abundance of these taxa. The greatest differences appear between the seasonal AASW and the two mesopelagic WW and mCDW water masses. Our results agree with results from previous surveys based on clone libraries (Gentile et al., 2006) or pyrosequencing of 16S rRNA genes (Delmont et al., 2014; Kim et al., 2014; Wilkins et al., 2012), but go further by considering separation by water mass in the mesopelagic zone. Normally, a variety of factors in combination shape marine bacterial community structure and create spatiotemporal distribution patterns (biogeography). In AASW for instance, factors known to control the population dynamics of bacterioplankton include light (this study), the availability of organic compounds (Sipler and Connelly, 2014), inorganic nutrients (Alderkamp et al., 2014; Sherrell et al., 2014) and complex interactions with eukaryotic phytoplankton blooms consisting mostly of *Phaeocystis antarctica* or diatoms (Delmont et al., 2014). Even if interactions between these factors ultimately shape bacterioplankton communities, individually they can cause different kinds of physiological responses in individual bacterial groups. Light, for instance, can induce a shift from heterotrophy to photoautotrophy, while eukaryotic phytoplankton may control resource availability by exudation of organic matter. While the present study focuses on bacterial population dynamics shaped by light and hydrographic separation, the overall mechanism of environmental gradients determining microbial biogeography in this region at the level of combined communities will be presented and discussed elsewhere. Future applications of metagenomic and metatranscriptomic techniques may also shed light on these issues.

Diversity in experimental treatments

In the incubation experiments, the origin of the seawater, serving as both inoculum and medium, explained most of the differences in the composition of emerging bacterial communities, while light exposure caused a modest inhibition of growth in all but the deepest water mass. Community richness after 7 days of experimental incubation was reduced compared to the bacterioplankton communities residing in the respective original water mass. This result is not surprising, as the preparation of dilution-extinction series for environmental samples is known to reduce diversity (Garland and Lehman, 1999).

Furthermore, particle-associated bacteria as well as larger grazers were removed from the seawater medium by pre-filtration, which will alter the overall diversity and activity in the experiments (Delmont et al., 2014; Williams et al., 2014). Grazing as a top-down regulating factor can, for instance, prevent copiotrophic populations from becoming dominant under resource-deplete conditions (Hahn and Höfle, 2001). Nevertheless, the community composition after incubation generally reflected the community composition in the original water masses, suggesting that grazing is of minor importance in controlling the bacterioplankton communities in this study. We cannot exclude that a longer incubation time may have allowed for additional shifts in community composition in response to the experimental manipulations; however, we chose a relatively short incubation period to minimize the risk of confinement effects (Massana et al., 2001). Mechanical disruption caused by pre-filtration of the seawater inoculum might also have affected the BCC or media composition. The changes observed in each of the three independent experiments were all in the same direction, with growth inhibited by light and greater richness observed in communities emerging from the mesopelagic water masses than from the surface water mass. This consistency of response implies a true treatment effect induced by light and not an indirect effect from active (or inactive) phytoplankton. The estimated growth during incubation was similar to that estimated previously for related polar ocean habitats (Rivkin et al., 1996).

Population dynamics across water masses

The *Flavobacteriales* genus *Polaribacter* contributed most to the emerging communities in the incubation experiments. This genus was dominant both *in situ* and after incubation for AASW and WW while present in much lower abundance in mCDW. Corresponding with its highest relative abundance in samples from the photic AASW, *Polaribacter* also appeared dominant in all incubations exposed to light, despite the removal of phytoplankton as a source of dissolved organic matter (DOM). Enhanced solar radiation has the capacity to hamper bacterial growth in surface waters (Sommaruga et al., 1997; Alonso-Sáez et al., 2006), a general effect also observed in our treatments. However, *Polaribacter* appears to benefit from light, either by direct phototrophic energy metabolism or by being more photoresistant than their competitors. The *Flavobacteria* contains bacteria that function as both free-living and particle-attached populations, is highly abundant in marine and freshwater environments, and has been linked specifically to the uptake and decomposition of polymeric DOM (Kirchman, 2002; 2008). Some *Flavobacteria* exhibit maximal growth rates that are nearly 2-fold higher than those of other abundant bacterial groups, resulting in pronounced growth during phytoplankton blooms (Zeder et al., 2009). Their abundant presence in the ASP region has been reported before (Kim et al., 2014; Delmont et al., 2014), as well as their increased activity during episodes of high primary production (Grzymski et al., 2012), presumably due to their extensive metabolic capabilities and photoheterotrophy (Williams et al., 2012). These findings are all consistent with the extraordinarily high productivity in the surface waters (AASW) of the Amundsen Sea Polynya (Yager et al., 2012; Williams et al., 2014).

The high abundance of *Polaribacter* in AASW and in light-exposed incubations could represent a seasonal population bottleneck, defined as a reduction of the gene pool of a community due to superior growth of one specific population in a community. Such bottlenecks typically happen during cultivation or in artificial laboratory conditions (Nei et al., 1975; Koskiniemi et al., 2012). However, in the present study the copiotroph *Polaribacter* appears to expand in an analogous way in the natural ecosystem, where it is as dominant in epipelagic waters as in the experimental incubations. Accordingly, this type of population bottleneck may reduce diversity in natural ecosystems, an explanation that is consistent with the lower estimated richness in AASW where *Polaribacter* contributed 50–70% of the total community.

Similar to *Polaribacter,* members of the genus *Balneatrix* (*Oceanospirillales*) are known to be present at high concentrations in photic AASW, often associated with intense phytoplankton blooms (Nikrad et al., 2013). The response of *Balneatrix* in the experimental incubations, however, differed from that of *Polaribacter*. *Balneatrix* was less dominant in the experiments independently of light conditions than in the upper water masses. An explanation for this response in the experiments could be, on the one hand, the lack of phototrophic energy metabolism in these heterotrophic bacteria and, on the other hand, the uncoupling from primary production and associated release of autochthonous organic matter in the experiments, as phytoplankton had been removed from the incubation medium.

Several other bacterial taxa remained in low abundance across all water masses and in the experimental incubations. Those populations are likely to have an oligotrophic lifestyle without the capacity for rapid proliferation. Members of the chemoheterotrophic *Ulvibacter*, for instance, belonging to the family *Flavobacteriaceae*, exhibited opposite abundance patterns to the closely related *Polaribacter*. *Ulvibacter*, previously isolated from the Southern Ocean (Choi et al., 2007) did not increase in abundance during our experimental incubations. Nevertheless, it was sometimes abundant, though highly variable in WW, possibly related to changes in local resources in this water mass. Isolates of *Ulvibacter* have gliding motility as a typical trait (Choi et al., 2007), indicating a particle-associated lifestyle. Hence, one possible explanation for the low representation of this group in the incubations, and variable abundance *in situ*, is that many of these bacteria were particle-associated and thus removed during pre-filtration of the seawater inoculum.

In general, the incubated samples, which originated from three different stations, responded similarly to light and by water mass source, yet the emerging communities varied in Simpson diversity and richness among stations, indicating that there was a site-specific response. For instance, the abundance of *Colwellia* after incubation varied by station. *Colwellia* is considered to be a genus of obligate psychrophiles involved in the production of extracellular polymeric substances required for biofilm formation, as well as enzymes for the breakdown of such high-molecular-weight organic compounds (Huston et al., 2004; Methé et al., 2005); *Colwellia* species are also known from sea ice communities (Bowman et al., 1998). This group of bacteria may be responsible for much of the important breakdown of export attributed to particle-associated, mesopelagic bacteria in the ASP (Ducklow et al., 2014; Williams et al., 2014), where *Colwellia* has been found associated with particulate matter (Delmont et al., 2014); such particle association may have led to selective removal of this group in the experimental pre-filtration. Chemoheterotrophic *Colwellia* have also been identified as being active in dark bicarbonate uptake (Alonso-Sáez et al., 2010), an intriguing possible contribution to their emergence in the dark treatments.

Response to light

The phototrophic *Roseobacter* lineage was a minor component both *in situ* and in the experimental incubations, even though it is one of the most abundant and well-studied clades of bacteria in the marine environment, where it can contribute up to 20% of bacterioplankton communities in some coastal regions (Buchan et al., 2005; Wagner-Döbler and Biebl, 2006). A number of metabolic traits have been identified in the *Roseobacter*, enabling members of this genus to interact closely with phytoplankton (Geng and Belas, 2010), including chemotaxis for motility towards free nutrients and fimbrial adhesins facilitating adhesion to phytoplankton. Coupled to the latter is a better opportunity to exploit DOM and organic sulfur compounds (e.g. dimethylsulfoniopropionate-DMSP) released from phytoplankton (Ruiz-González et al., 2012; Tortell et al., 2012). In the ASP, *Roseobacter* was rather scarce and, despite being known to share many metabolic and ecological traits with *Polaribacter*, was apparently not competitive in any of the incubation experiments. The ability of *Roseobacter* to adhere to particles and thus be removed during pre-filtration may have contributed to these results.

Light can have contrasting effects on different populations of bacteria. Light-inhibition was observed for the *Gammaproteobacteria* clade SAR92 in WW. This result seems to contradict earlier findings, where isolates of this group tested positive for the light-driven proton pump proteorhodopsin and were considered oligotrophic (Stingl et al., 2007). Our apparently contradictory result emphasizes that the presence of individual genes, as identified in isolates, does not always correlate with functional responses *in situ*. Isolates related to the abundant SAR92 clade found in the ASP have also been characterized as members of the oligotrophic marine *Gammaproteobacteria* (OMG) (Cho and Giovannoni, 2004), indicating a predominantly oligotrophic life strategy in the pre-bloom WW. Within clade SAR86 (*Gammaproteobacteria*), some subgroups carry proteorhodopsin and display enhanced growth when exposed to light (Schwalbach et al., 2005). Nevertheless, this clade did not respond to light treatments. This result is not surprising, given that SAR86 in the ASP was mainly abundant in the dark water masses and in experimental incubations originating from dark waters, and thus not likely to rely on phototrophic energy metabolism.

The ubiquitous *Alphaproteobacteria* clade SAR11 from different samples responded in contrasting ways to the experimental incubations, but rarely responded to light. SAR11 is known to prefer low molecular weight and labile organic compounds and also to carry proteorhodopsin (Giovannoni et al., 2005; Tripp, 2013). Interestingly, we detected a much lower abundance of SAR11 compared to Kim et al. (2014) or Delmont et al. (2014), which might be related to different sampling conditions or the application of different primer pairs, though an explanation for this difference is not obvious.

Conclusion

This study presents an example of how environmental heterogeneity affects bacterial community composition in the ASP. We show in particular how hydrographical separation accounts for shifts in the abundant fraction of taxa, which we attribute to differences in bacterioplankton population dynamics between the water masses driven in part by light availability. The BCC in the photic AASW was characterized by low richness, favoring dominant bacterial populations related to taxa known to expand under bacterioplankton bloom events. In contrast, the BCC in the mesopelagic water masses had higher richness, featuring taxa known to benefit from oligotrophic conditions. Grazing appeared of minor importance, whereas (in hindsight) our method of pre-filtration may have removed an important fraction of particle-associated bacteria. Incubation experiments indicated the importance of light as a regulating factor of the BCC: light in general inhibits community growth, but some populations, particularly *Polaribacter* in the ASP, profited directly from light conditions and increased in abundance. In parallel with the strong influence of phytoplankton and their exudates, our experimental treatments help to explain how changing light conditions in surface waters, with the seasonally variable ice conditions, may influence the dynamics of BCC between photic surface and dark mesopelagic waters.

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Contributions

- Contributed to planning and experimental design: SB LR IR
- Contributed to acquisition of data: IR JD RL PY
- Contributed to analysis and interpretation of data: IR JD RL AW LR SB, PY
- IR wrote the paper with the help and inputs from all coauthors

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Competing interests

The authors have declared that no competing interest exists.

Data accessibility statement

Accession No.: PRJEB4866 (European Nucleotide Archive).